3. Blood Tests after Smoke Treatment *

To supplement the morphological findings in smoke-exposed and not smoke-exposed gold-hamsters, we plan to discuss in the following, on hand of the available data, the question of the influence of smoke-substances on different biochemical and hematological blood values. Particularly in the evaluation of vascular processes, but also in the interpretation of hematological findings, a knowledge of the influence of the smoke treatment on the hematogenesis, on enzyme systems and other factors seems significant.

An abundance of literature is available on the influence of smoke-substances on the metabolism in man. The results were summarized in the two reports by the SURGEON GENERAL I97I and I972 as well as in an address by SCHIEVELBEIN and EBERHARDT (I972). In this summary are included numerous reports concerned with the problem of the influence of smoke-substances on blood coagulation, whereby SCHIEVELBEIN and EBERHARDT (I972) state that " a statement on the influence of nicotine on coagulation can hardly be made at the present time".

Material and Methodology:

The tests were carried out according to the initially described methodology. We used hamsters from the breed Zucca (USA) and from the breed Coombehurst(CMH) (England). The animals were accepted into the test after the usual quarantine. The age of the animals at test begin and the pertaining test length are listed in the charts I00 - I33. A further condensation of the charts was not possible according to statistical consideration because

a) the animals came from different colonies
b) the test times in respect to duration and the season were varied
c) the cigarettes with the same tobacco mixture were not completely identical because of different pesticide additives. The smoke-treatment was carried out according to the initially described method. The blood tests were made at the same time of day in all test animals between I0 - 20 min. after finished smoke-treatment. Blood for the counting of leukocytes and erythrocytes etc. was taken from the retro-orbital plexus of veins, the remaining examinations were made with the total blood of the hamster. The Blood-letting was done after light ether narcosis by severing the aorta in the area of the thorax. The examinations of erythrocyte numbers, leukocyte numbers and hemoglobin values, lasting to the 56 th life-week, were undertaken on chronically smoke-exposed animals of group 5, respectively on animals of the control group K which were selected at random. From these animals, blood was taken under light ether narcosis from the retro-orbital plexus of veins. After the extraction of blood, the animals were returned to their respective groups.

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For the smoke-treatment we used, as a rule, the standard cigarettes 3. In a part of the test results, the tests had been made with cigarettes which contained an admixture of pesticides. We included the values for these animals in order to facilitate a better survey on a larger material. The results of the pesticide tests will be reported at another time, they show no influence on the blood findings. The basic tobacco mixture of these cigarettes was equivalent to the standard cigarette.

The methods for the conducted tests are compiled in chart 99. For all values, we established the average value as well as the standard deviation and listed the range. The listing of comparative literature enables the reader to recognize the dispersion of the values in individual testing methods which is considerably higher for the goldhamster than for other test animals (e.g. rats).

Results:

The results of the blood tests are compiled in the charts I00 - I34. The use of hamsters from different colonies with, in parts, differing ages of the animals as well as conducting the tests at differing times, particularly seasons, and with differing numbers of animals, does not permit to consider all animals of the 3 groups (smoke-exposed group, fresh air controls, untreated controls) mathematically together, since, statistical differences are, in parts, already existent within the 3 groups. With exception of the erythrocyte values, the leukocyte values and the hemoglobin values for which we conducted an extensive statistical evaluation, we therefore limited ourselves in respect to the remaining biochemical data and blood values to the computation of the average values, the standard deviations and the listing of the range.

The findings which we obtained on a large stock of animals essentially correspond to values found by other authors. In comparison to pathological values, as they have been found in certain disease conditions and organ changes, the average values, the standard deviations as well as the range rest within the normal area for smoke-exposed and not smoke-exposed animals. Values which deviate distinctly from the norm are mentioned separately in the discussion of the individual data.

a. Hemoglobin

LARSON and SILVETTE (1968) compiled the reports which were concerned with the influence of smoke-substances on hemoglobin (Hb). According to them, BLACKBURN and Assoc.(I960)

reported that the Hb does indeed show a tendency to increase in smokers, that it is not significant though. The remaining literature data found by LARSON and SILVETTE (1968) do also not show an unequivocal increase of Hb in smokers in comparison to non smokers. Experimental tests with animals dealing with the problem of the influence of smoke-treatment on Hb are not known to us.

Chart 100 shows the obtained Hb-values for control animals and smoke-exposed animals. The animals of group 5, smoke-exposed for over one year in chronic smoke-treatment tests were statistically compared to the pertaining control groups K. The result of the variance analysis shows a strong concurrence with the result of the statistical calculation for the erythrocytes. Here too, factors became evident which influence the Hb-value in a significant manner:

a) the treatment whereby the smoke-exposed animals showed higher values ($p < 1\%$),

b) the sex of the animals whereby the male animals, independent of the treatment, showed higher hemoglobin values ($p < 1\%$),

c) of the interreactions, that between smoke-treatment and weeks is significant ($p < 1\%$), that is, the influence of the treatment varies according to different weeks. The total influence of the treatment is 0 at 20 weeks for the smoke-exposed animals as compared to the controls, it rises up to the 44th week and is smaller again at 56 weeks.

d) the interreaction between treatment and sex is still contingent on coincidence.

The obtained values (chart 101) show a vast concurrence with the numbers stated in literature. The present experiment shows an unequivocal influence of the smoke-treatment on the Hb-quantity only with a long test duration.

b. Erythrocytes

In earlier experiments (REICHEL and DONTENWILL 1970), we were concerned with the influence of high-dosed smoke-treatment on the number of erythrocytes in the hamster (Colony "Seeboth").
With prolonged smoke-treatment (over 100 days), we saw an increase of the erythrocytes from 5.4 to 6.9 millions. This increase became even greater after a smoke-treatment length of 143 days and reached a ratio of 5.5 : 7.9 millions. We attributed the increase of erythrocytes to the chronic CO-intoxication which is unavoidable in the chosen tes

arrangement for the chronic smoke-treatment. In earlier experiments (REICHZEH and Assoc. 1969), we dealt in detail with the strong increase of the CO-Hb in smoke-exposed hamsters. Through the interval-free, long lasting exposure to smoke, the CO-Hb level rises considerably stronger than this can be observed in the smoking habits of man, where each puff is followed by a greater interval. We have not been able to find other experimental tests with animals on the influence of chronic smoke-treatment on the number of erythrocytes.

Chart I02 shows, in the comparison between test animals and controls, the increase of the number of erythrocytes in the smoke-exposed animals, their strength is dependent on the duration of the smoke-treatment. The compensatory increase of the erythrocytes, produced by the chronic CO-intoxication, is only understandable through the test arrangement and the increase of CO-Hb which it causes. However, this compensatory increase of the erythrocytes also shows that the animals are exposed to a strong CO-influence during the smoke-treatment and that this factor therefore has an effect which is multiply greater than it is in man during smoking. The findings as well as the quantitative examinations of the smoke-intake allow for the conclusion that the animals absorb considerable quantities of smoke-substances in the chosen test arrangement. The animals of group 5, smoke-exposed for over one year in chronic smoke-treatment tests, where statistically compared to the pertaining control groups K. In a variance analysis of the tests, we found as factors which influence the number of erythrocytes in a significant manner:

a) the treatment, whereby the smoke-exposed animals showed higher values ($p < 1\%$),

b) the sex of the animals, whereby the male animals, independent from the treatment, showed increased numbers of erythrocytes ($p < 1\%$),

c) of the interreactions, that between smoke-treatment and weeks is significant ($p < 1\%$), that is, the influence of the treatment varies with varying numbers of weeks. The total influence of the treatment is 0 at 20 weeks for the smoke-exposed animals as compared to the controls, increases up to 44 weeks and is less at 56 weeks.

d) the interreaction between treatment and sex is still contingent on coincidence.

The obtained values show a vast concurrence with the numbers stated in literature (chart I03).

c. Thrombocytes, Prothrombin Time, Hematocrit Value

It would lead too far to discuss all the reports which deal with the influence of smoke-substances on blood coagulation, the agglutination of thrombocytes, respectively the factors of blood coagulation.

In his condensation, MURPHY (I968) writes about the thrombocytes, the thrombosis and the coagulation : "it is possible that smoking shows an acute effect, the formal proof is so far still lacking". Extensive literature can be found by LARSON and SILVETTE (I968) as well as by SCHIEVELBEIN and EBERHARDT (I972). They are almost exclusively tests, respectively observations on man.

Chart I04 shows that the number of thrombocytes is not unequivocally changed by smoke-treatment. However, the larger dippersion and range of the thrombocyte numbers is conspicuous in the smoke-exposed animals.

To supplement this, we would like to point out that BIRNSTINGL and Assoc. (I97I) found after treatment of rabbits with CO (400 ppm), a strong decrease of the number of thrombocytes (before 57, 5I per cmm - afterwards 33, 46) while at the same time the adhesiveness decreased from 30.04 % to 24.08 %.

The prothrombin time shows no change between smoke-exposed animals and controls. An influence on this coagulation factor is not recognizable (chart I05).

As can be recognized in a comparative observation of the hematocrit value and the number of corpuscular blood components, the hematocrit value rises parallel to the increase of erythrocytes (chart I06).

d. Leukocytes

An influence of nicotine on the number of leukocytes was studied by various authors (see LARSON and SILVETTE I968). MINUTH (I959) saw in smoke-treated rabbits one leukopenia, one granulocytopenia as well as an increase of the eosinophile leukocytes. Earlier in comparative experiments, we (REICHEN and DONTENWILL I970) had not been able to detect an unequivocal increase of the leukocytes after chronic smoke-treatment.

In an epidemiological study, CORRE and Assoc. (I97I) found an increase of leukocytes in smokers as compared to non-smokers. The differential blood-count did not show any essential changes. ASANO and Assoc. (I962) could prove that, after the smoking of 5 cigarettes, the total number of leukocytes rises parallel to the increase of the adrenaline level in the urine. He designated this as a "stress"situation. (Name illegible, Tr. and CASTRICIANO (I968) were able to prove an effect on the leukocytes in the periphery after the inhalation of cigarette smoke. In context to the question of a triggering of allergic reactions through smoke substances, SCHOEN and PIZER (I964), SCHRODER (I967) and SAVEL (I970) reported casuistic findings. They observed changes of the leukocytes in allergies, respectively tried to study, by means of different testing methods, the significance of tobacco substances for changes in the blood-count. The available findings do not give a uniform picture.

In chart I07 are compiled the total leukocyte numbers for the controls and the smoke-exposed animals. Chart I07 does not show any unequivocal differences between smoke-exposed animals and controls. We find a minor decrease of the leukocyte number in the smoke-exposed animals which runs parallel to the decrease in body-weight of these animals. In one test on not smoke-treated animals with reduced feeding, we were able to prove, as chart I08 shows, that a reduction of the body-weight by 36 % within 4 weeks also results in a decrease of the leukocyte numbers by 35 %. Since reductions of the numbers of leukocytes have been described in man upon a strong reduction of the body-weight (hunger) (VILLINGER-KNERCH I957), we assume that this factor was also decisive in the present case. In a statistical analysis of the chronically smoke-exposed animals of group 5 with the pertaining not smoke-exposed control animals of group K, we can regard as significant ($p < 5$ %), the interreactions of treatment and sex and the joint interreaction of treatment, sex and weeks. The total effect of the treatment is the summ of these effects: it presents for the smoke-exposed animals compared to the control animals an increase for the male animals, a reduction for the female animals of the values; this effect is more strongly pronounced in the 20th and 56th week than in the 30th and 44th week. These, in parts, high fluctuations of the numbers of leukocytes is on the basis of our own examinations not at all explainable through the presence of infects; a possible influence could be exerted by seasonal factors. The differential blood-count of the leukocytes shows a relative prevalence of the lymphocytes, a finding which is equally established by all researchers. An unequivocal influence of the smoke-

treatment on the differential blood-count is not recognizable (chart II0, II2, II4, II6, II8).

A comparison to the findings or other authors is offered by charts I09, III, II3, II5, II7, II9.

e. Reticulocytes

Tests on the influence of smoke-substances on the reticulocytes are not available. Chart I02 shows that the average values and standard deviations between the smoke-exposed animals and the control groups, as well as the range, rest within the normal area. The low reticulocyte numbers in 2 or 4 control groups (groups 38 and 39) can be caused by the low age of the animals and/or by the season. RATHS (1957), for instance, found low reticulocyte values in summer (chart I20).

f. Electrolyte, Total Protein

We purposely carried out the determination of the electrolyte as well as the determination of other biochemical factors (e.g. total protein) within in the first 6 months of the life of the animal. The reasons rest in the increasing frequency and severity of the amyloidosis of the kidney in the goldhamster. Since, with increasing amyloidosis and thereby caused nephrosis, electrolyte shifts and changes of the blood protein consistency are conceivable, the question of the total protein and the electrolyte determination seemed more significant to us with an intact kidney parenchyma . Charts I2I - I23 show the obtained values for total protein and electrolytes. The electrolyte values and the total protein show, with the animals treated with fresh air also included in the consideration, no significant difference between smoke-exposed and not smoke-exposed animals.

A comparison with the findings of other authors is shown in charts I22 and I23.

g. Total Bilirubin

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In order to clarify the influence of smoking on the functions of the liver, we determined besides enzymes the total bilirubin in the serum. No unequivocal differences are found between smoke-exposed animals and controls. The minimally lower values of the groups 38 and 39 in the untreated control animals may be caused by the low age of the animals (chart I24).

h. Enzymes: alkaline phosphatase, glutamate-oxalacetate-transaminase (SGOT), glutamate-pyruvate-transaminase (SGPT).

No information can be found in literature on the influence of smoke-substances on these enzyme values in the blood serum. It is conspicuous that in respect to SGOT in the hamster, a distinct sex difference is provable in individual groups. As the chart I25, in which the values are listed separately for the sexes, shows clearly, the average values for the male animals are, independent of the treatment, approximately twice as high as those for the females. Differences between treated and not treated animals cannot be proven (chart 126). How strongly the SGOT-values can rise, for instance, with infections (entamoeba histolytica), is demonstrated by the findings of RAETHER and Assoc. (1967). The SGOT-values rose from 42.0 (measured at room-temperature) after infections to maximally 4540.0. SGOT-values which were determined at 35 °C in the auto-analyser, after the method applied by us, are found by OPIE and Assoc. (1964). They found values of II7 ± 27.

The SGPT-values and the values of the alkaline phosphatase show no differences between treated and untreated animals (charts I27 and I28).

i. Total Cholesterin, Free Cholesterin, Esterized Fatty Acids, Free Fatty Acids, Neutral Fats

We have already refered to the significance of the lipoid metabolism for the formation

of arteriosclerosis in the discussion of heart and circulatory diseases.

Numerous researchers have concerned themselves with the question of the influence of smoke-substances on the lipoid metabolism. A combination of these experiments is given by the reports I97I and I972 of the Surgeon General, as well as by the monography by LARSON and SILVETTE (I968) and by the report by SCHIEVELBEIN and EBERHARDT (I972). Th findings which were obtained in animal experiments are considerably more varied than the findings obtained in man. In man (SCHIEVELBEIN and EBERHARDT , I972), a significa raised cholestrin level was found in healthy smokers, respectively through the inhala of smoke-substances an increase of the free fatty acids.

We compiled the results of our tests in the charts I29 - I33.

Free cholesterin, esterized fatty acids, free fatty acids and neutral fats do not sho any differing values in the treated and untreated animals, under consideration of the varied arrangements. For the total cholesterin, an increase in the treated animals is not provable. Under consideration of the strong dispersion of the values, a decrease of the total cholesterin value in treated animals is not evident. How strongly the total cholesterin can rise, is demonstrated by feeding experiments (cholesterin) by SELIUK and BEHER (I969) who saw an increase from I38 ± 6.4I to 35I ± 57.4 and by WELL and ERBSHOFF (I962) who saw, with the same test arrangement, an increase from I32 ± 7 to 495 ± 32.

Altogether, the present biochemical findings of the serum and the hematological data after acute, subacute and chronic high-dosed smoke-treatment, show, that unequivocal changes were provable only for the erythrocytes and the hemoglobin. Both show a statistically significant rise which was considered to be a consequence of the test conditions (chronic CO-effect). The decrease in leukocyte values, proven in two groups, was only evident in female animals; it is possibly the result of the simultaneous decrease in body-weight caused by the smoke-treatment.

4. Comparison of Doses

In order to facilitate a comparison between the active smoking of man and the smoke-treatment of test animals, particularly however, to make a relation between dose and effect possible, a few explanatory data shall be cited.

The present-day smoke-norm (Coresta Standard No. IO)which also corresponds to the German Industrial Norm (DIN IO 240 - I969), is based on the assumption, that the smoker in the average smokes with a puff-volume of 35 ml., a puff-length of 2 sec. and a puff-frequency of I puff/min.

The approximately 35 ml smoke taken in per puff into the oral cavity can be exhaled by the smoker with the next breath without any previous inhaling, or he inhales with a new breath of variable quantity and therefore depth this smoke-quantity into the "breathing ducts". The average volume per breath is 500 ml. The "breathing ducts" would thus contain a smoke/air mixture of 35 : 465 ml. Approximately I50 - 200 ml fall to the share of the dead-air space. With the following expiration a part of the smoke/air mixture, particularly from the dead-air space, is exhaled without having reached the bronchi or the alveoli. A part remains in the respiratory tract depending on the depth of inhalation. A near I00 % intake of the smoke-substances is therefore impossible.

In our tests, we are essentially interested in the substances of the particle-phase of the smoke. Contingent upon the smoking habits of man inhaling via the oral cavity, we can assume, that, despite full inhalation, only approximately half of the smoke-particles remain in the respiratory tract, since, as already discussed, a part is exhaled and another part is transported away by the lung clearance.

Since there is a pause of 58 sec. between each puff of 2 sec., we have a relatively favourable situation for the removal of absorbed smoke-particles through exhalation and lung clearance.

For the animal the conditions in our test arrangement are totally different. The animal breathes 3 x daily, IO min each time, from a vacuum-free system interval-free smoke/air mixture with a mixture ratio of I : I5. After a few breaths the lung contains only an equivalent smoke/air mixture. Since always the same mixture is offered, there are no puff pauses and therefore no purifying exhalation or time intervals for lung clearance. Under physiological conditions, the hamster has a breathing frequency of 75 breaths/min. On the basis of our own experiments (RUECKER and DONTENWILL I967), the frequency increases within the smoke-treatment period. If we take as a basis I00 breaths per minute during the smoke-treatment, the hamster inhales in IO min. approximately I000 puffs smoke/air mixture with a vast succumbing of the lung clearance. Because of the different flowing conditions, in contrast to man, in the area of the upper respiratory tract of the goldhamster, the strongest deposition of smoke-particles per unit of area takes place in the larynx. This concentration is approximately 300 x as great as in the lung area(DONTENWILL and Assoc. I97I, CHEVALIER and DONTENWILL I972). Since the dose per area is decisive for the effect, these factors receive a great significance. Furthermore, it must be considered that in the region of the cilia-free larynx parts, a purification is less easily possible and that the pavement epithelium reacts possibly more sensitively than the bronchial or tracheal cylinder epithelium (DONTENWILL and MOHR I962 b).

The evaluation of all factors shows that it is near impossible to establish an exact dose comparison between man and the animal, but that the effective dose on the larynx of the animal certainly constitutes a multiple of the dose effective in man per unit of area in the respiratory tract.

These calculation do not touch on the comparison between two test groups with the same methodology which is intended to show which dose or cigarette modification has a stronger or weaker effect.

The factors which lead to the highly applicated smoke quantity in the animal, strengthen to a particular extent the resorption of compounds which are absorbed from the smoke on account of an active participation of the body. This fact must be pointed out separately. The circumstances shall be discussed in more detail on two special components of smoke.

Carbon monoxide (CO) on account of its great affinity ~~of~~ to hemoglobin is greatly favoured in its absorption from the smoke/air mixture which is permanently offered to the animals for breathing during IO min. This makes the CO-Hb-value of the smoke-exposed animals understandable which rests near the toxic dose (RECKZEH and DONTENWILL I970). The CO-Hb-level is probably after each smoke-treatment considerably higher for longer periods of time than in the strong smoker (DONTENWILL and Assoc. I966). In the hamster, we must therefore also expect changes in the course of the smoke-treatment experiment, as they have been found by THEODORE and Assoc. (I97I) in various animals with nothing but CO-inhalation. This is evident in the present experiments with the hamster on the values of the erythrocytes, the hemoglobin and the hematocrit.

Nicotine is also faster resorbed from the smoke than other substances like for instance hydrocarbons, and is quickly transported away (DONTENWILL and Assoc. I97I, ISAAC and RAND I969). This facilitates a further resorption and the lung clearance can only have a limited effectiveness for the nicotine. Moreover, nicotine can also be resorbed in the area of the cavity of the nose and the oral cavity. We must therefore assume that the animals take in a high quantity of nicotine.